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Leaching capacity of a new extremely thermophilic microorganism, *Sulfolobus rivotincti*

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Abstract

The leaching capacity of a new extremely thermophilic microorganism (*Sulfolobus rivotincti*) was tested on different metal concentrates. The results using three complex sulphide concentrates demonstrated, in all cases, the culture's capacity to recover high percentages of copper and zinc with slight differences according to the composition of the concentrate used. The bioleaching of chalcopyrite concentrates was studied in the presence of different compounds (chlorides and metallic ions) in an attempt to improve the efficiency of bioleaching. The results confirm the high toxicity of the chloride ion and the negative effect of silver. Of the other ions studied, only bismuth had a positive effect, increasing the rate of copper leaching, although the final percentage of metal in solution was very similar to that obtained with experiments not involving bismuth.

Keywords: *Sulfolobus rivotincti*; Leaching; Thermopilic bacteria

1. Introduction

Industrial bioleaching systems usually use mesophilic microorganisms, particularly autochthonous bacteria associated with the mineral under attack. However, residence times are very long and it is only economically viable to treat low-grade ores. Hence, any reduction of the leaching time is interesting from an economic point of view. One of the solutions proposed by researchers has been the use of thermophilic microorganism strains. Thermophilic archaeobacteria, for example, with an optimal growth temperature of between 60 and 80°C have been the subject of several recent studies because of their

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capacity to solubilize a number of metallic sulphides [1–4]. The results obtained seem to demonstrate a greater efficiency than that obtained using mesophilic microorganisms such as *Thiobacillus ferrooxidans* for similar processing times [5].

Thermophilic archaeobacteria possess a series of characteristics which makes them especially suitable for bioleaching or biodesulphurization of coal since they increase the speed of dissolution of metallic sulphides as a consequence of a higher reaction temperature. In addition, they are capable of dissolving ores such as molybdenite and chalcopyrite which are difficult to attack using mesophilic microorganisms. The tests carried out with bacteria of the genus *Sulfolobus* have provided high copper extraction yields from chalcopyrite. However, the degree of effectiveness is not the same for all microorganisms of this genus and varies from one species of *Sulfolobus* to another [6,7]. Thus, it is important to find new species capable of giving high dissolution percentages when used with this type of ore. Thermophilic bacteria also have disadvantages as a result of the greater sensitivity of their cell walls at high pulp densities and their lower tolerance to toxic metals than mesophilic microorganisms.

Within the thermophilic archaeobacteria, the most widely used have been different species of the genus *Sulfolobus*, especially *Sulfolobus acidocaldarius* or *Sulfolobus* BC [8,9], and *Acidianus brierley*. However, during recent years new species of thermophilic microorganisms with a greater capacity to dissolve metallic sulphides have been isolated and characterised, for example, *Metallosphaera sedula* [10], *Sulfolobus metallicus* [11] or *Sulfurococcus* [12]. At the Complutense and Autónoma Universities of Madrid, a new species of the genus *Sulfolobus*, termed *Sulfolobus rivotincti*, has recently been isolated and characterised. During preliminary experiments, this microorganism, which was isolated from samples taken from the mining area of Rio Tinto (Huelva, Spain) has shown a high capacity to dissolve metallic sulphides and a strong resistance to different toxic metal cations. The present contribution discusses the leaching characteristics of this new species of *Sulfolobus* when exposed to different metal sulphide concentrates from mines in the South of Spain.

2. Experimental and methods

A new pure culture of the newly isolated and characterised thermophilic microorganism *S. rivotincti* was used [13]. Differential and bulk concentrates of complex sulphides were obtained from mines in Aznalcollar and Rio Tinto (Spain). The chalcopyrite concentrate was taken from Rio Tinto mines. The principal mineralogical phases and the composition of the concentrates are shown in Table 1. The minerals were sterilised by dry heat (140°C for 24 h) before being used in the experiments. The microorganisms were cultured in 250 ml shake flasks using an orbital incubator with a stirring speed of 150 min^{-1} and a constant temperature of 68.5°C. 9K medium (95 ml) [14] without iron at pH 2 was used as culture medium. Several mineral concentrates at different pulp densities were used as energy source. The tests were inoculated with 5% (v/v) of a previous culture grown on the same mineral concentrate which was used in the subsequent experiments. During each experiment, a series of samples was taken from the leaching solution after letting it rest for a short time without being stirred so that the

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Table 1
Concentration (%) of the principal metallic elements and mineral phases in the concentrates used in the experiments

Mineral concentrates				
Metal (%)	CCu (Aznalcollar)	CCuL (Río Tinto)	CGRT (Río Tinto)	D1 (Río Tinto)
Cu	16.1	15.7	14.0	27.6
Fe	31.9	17.1	25.0	34.2
Zn	7.3	5.5	17.1	0.6
Pb	4.8	n.d.	1.7	n.d.
Mineral phases				
	(1) $CuFeS_2$	(1) $CuFeS_2$	(1) $CuFeS_2$	(1) $CuFeS_2$
	(2) Cu_2S	(2) FeS_2	(2) ZnS	(2) FeS_2
	(3) FeS_2	(3) ZnS	(3) FeS_2	
	(4) ZnS			
	(5) PbS			

The mineral phases are numbered in order of importance (n.d.: not determined).

suspended solid could be decanted. The total concentration of copper, zinc and iron in these samples was determined by atomic absorption spectrophotometry. The Fe (II) content was determined by photocolorimetry with orthophenanthroline [15] and Fe (III) was calculated by difference. The number of cells in each sample was determined by direct counting using an optical microscope with a Thoma chamber. Different amounts of solid NaCl were added to the flasks containing 9K medium and mineral until concentrations of between 0.005 and 1 M NaCl were obtained.

2.1. Leaching in the presence of different metallic cations

Different metals were chosen according to previous studies [16–18] on the leaching of metallic sulphides and chalcopyrite concentrates using thermophilic and mesophilic microorganisms. The tests were carried out in the presence of different cations (cobalt, mercury, arsenic, molybdenum, bismuth, chromium, aluminium, silver and cadmium) by adding the corresponding metal salts to the reaction medium ($CoSO_4$; $HgSO_4$; AsO_2; $Na_2MoSO_4 \cdot 2H_2O$; $Bi(NO_3)_3$; Ag_2SO_4) or suitable volumes of the commercially available solutions in the case of Cr, Cd and Al. As controls, a reference test with inoculum but without cation and a sterile test were used. All metals were added at a rate of 1 g metal (kg concentrate)$^{-1}$.

2.2. Leaching in the presence of silver and bismuth

These two cations are of special interest in the leaching of chalcopyrite [19–21] and thus a more thorough study of their use with *S. rivotincti* was made. 250 ml Erlenmeyer flasks with 90 ml of 9K medium, 1 g of chalcopyrite concentrate and the corresponding concentration of the cation under study were used. The concentrations of bismuth were 1, 5 and 10 g (kg concentrate)$^{-1}$. Since silver is more toxic to microorganisms the

concentrations of this cation were 1, 3 and 6 g (kg concentrate)$^{-1}$. After 1 h of chemical conditioning, 10 ml of a pure culture of the thermophilic microorganisms *S. rivotincti* were inoculated.

3. Results and discussion

3.1. Choice of the optimal pulp density

A series of experiments was carried out to determine the most suitable pulp density for subsequent bioleaching experiments. The biological treatment of ores could only be carried out at lower pulp densities than conventional chemical treatment. Several studies have pointed out the possible causes of this limitation citing the growth of toxic products in the medium, the lack of available carbon dioxide and oxygen [22] and attrition problems with the mineral particles. This last problem assumes special importance in thermophilic microorganisms due to the cell wall structure which lacks peptidoglicane and cell membranes, which are much more fluid than those of mesophilic microorganisms [23]. This means that this type of microorganism can tolerate only low pulp densities, which is one of the main problems for its industrial use [5,24]. Usually, bacteria of the genus *Sulfolobus* have been used with pulp densities of around 1% [6] although, after fairly long periods of adaptation, the amount of ore added to the pulp can be increased substantially [25]. The amount of ore also varies with the type of reactor used; the pulp density which can be used in air-lift reactors without inhibiting the growth of thermophilic microorganisms is greater than in mechanically stirred reactors. The mineral used for determining optical pulp density was a CCuL concentrate, on which the bacteria had been isolated and on which the highest cell populations were detected in previous bioleaching experiments. Flasks containing 9K medium without iron were prepared with different concentrations of solid (1, 2, 3, 5, 7 and 10%). No type of previous adaptation to the different pulp densities was made and the inoculation was carried out with bacteria from the maintenance tests. The result of these experiments corresponding to the final cell population after 20 days is shown in Fig. 1.

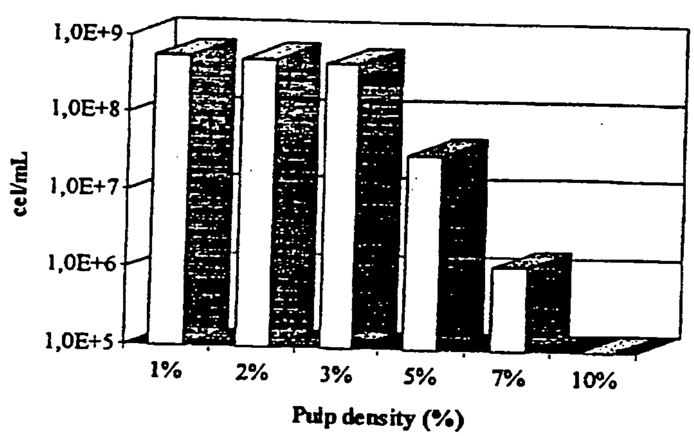

Fig. 1. Cell growth of the microorganism *Sulfolobus rivotincti* at different pulp densities.

Concentrations of 1 and 2% gave rise to the greatest cell growth although there was not much difference between these and the 3% concentration. Since the objective was to process the greatest amount of ore possible, it was decided to use a pulp density of 3% for the leaching tests. It must be borne in mind that the maintenance culture grew in a 1% pulp density and so the microorganism was well adapted to this concentration; however, it is also possible to carry out an adaptation process to higher densities in order to increase yields. With an ore concentration of 5% the cell population initially remained constant although subsequent decrease led to its disappearance in 15 days. Above 7% no cell growth took place, possibly because of attrition phenomena with the ore and lack of adaptation.

3.2. *Kinetics tests using different mineral concentrates*

After confirming the capacity of *S. rivotincti* to use ores of metallic sulphides as energy source and determining the optimal pulp density, a series of kinetic experiments was carried out to verify its performance in leaching processes. Laboratory bioleaching experiments which evaluate the use of a certain bacterial species on a given concentrate have a serious drawback in that the results cannot easily be compared with those of the literature since each concentrate has its own mineralogical characteristics. For this reason, three concentrates of different composition and geographical origin in order to add objectivity to the assessment of *S. rivotincti* leaching capacity were used. The concentrates used were CCu, CCuL and CGRT. Although the proportion of copper in all of them was similar, the amount of other elements, mainly zinc and iron, varied considerably. The experiments were carried out in 95 ml flasks containing 9K medium at pH 2 and 5 ml of inoculum. The temperature was 68.5°C and the flasks were stirred at 100 min^{-1}. Controls in sterile conditions were used to differentiate bacterial activity from chemical leaching which may be important at high temperatures. The results for metal dissolution in this series of experiments are depicted in Fig. 2.

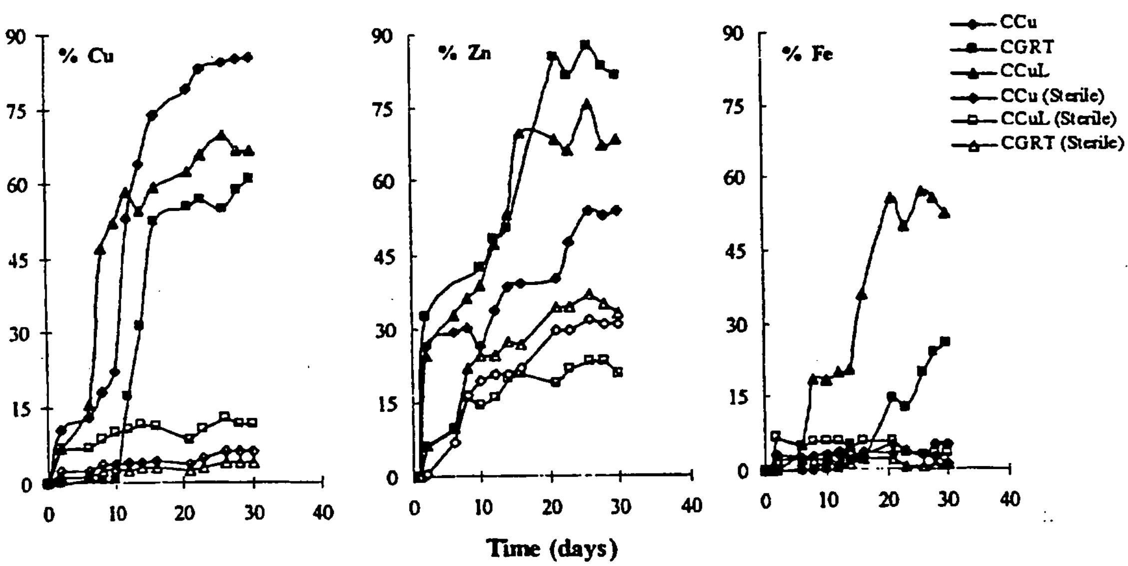

Fig. 2. Copper, iron and zinc dissolution from the three mineral concentrates used.

The amount of metal extracted from all the concentrates used was high and always greater than that obtained in the sterile tests, especially in the case of copper. Differences in the concentration of metal in solution were due to the type of concentrate with the best yields being obtained with the CCu concentrate in which chalcopyrite is associated with pyrite and the two mineralogical phases generate a galvanic couple which favours the dissolution of the chalcopyrite/pyrite association. The lowest concentration of dissolved copper was obtained with the CGRT concentrate, which can be explained by the absence of the chalcopyrite/pyrite galvanic couple and by the fact that the bacteria need a longer adaptation period with this concentrate. The increase in pH due to the consumption of acid by the gangue associated with the mineral (Fig. 3) also caused unfavourable conditions for bacterial development. Only when the bacteria began to grow (Fig. 3) and pH value decreased (7–10 days into the experiment) the amount of copper and iron in solution began to increase.

Zinc, on the other hand, was leached rapidly from the very beginning since ZnS is easier to attack chemically than chalcopyrite or pyrite so the intervention of bacteria is not so crucial. This was evident in all the concentrates tested, in which the concentration of zinc in solution increased from the first day. The preferential leaching of zinc over copper at high temperatures has been recorded by other authors and is attributed to galvanic interactions [26]. The product of this chemical reaction dissolving the zinc is elemental sulphur which can be used by the bacteria as energy source. The result is an increase in bacterial population, lower pH of the medium and optimal growth conditions, all of which favour the direct attack of the chalcopyrite present in the mineral:

$$ZnS \rightarrow Zn^{2+} + S^{0} + 2e^{-} \qquad (1)$$

$$2S^{0} + 2H_2O + 3O_2 \xrightarrow{\text{bacteria}} 2H_2SO_4 \qquad (2)$$

Since the shortest microorganism adaptation times correspond to the CCuL concentrate, the maintenance culture had been carried out on this concentrate. Moreover the

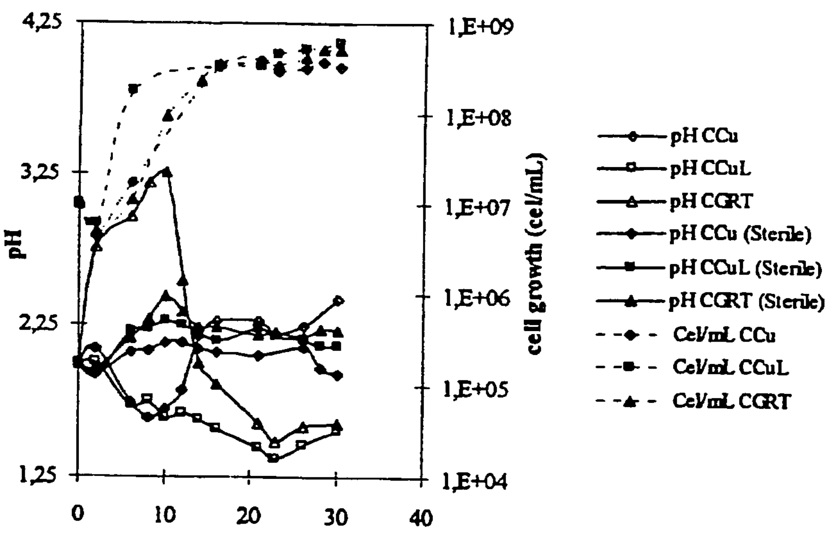

Fig. 3. pH and bacterial populations during the bioleaching of the three concentrates used in the leaching tests.

cell population reached its maximum most rapidly and the pH dropped most rapidly in this culture than when the other concentrates were used. The ferro-oxidizing behaviour of the microorganism can be seen clearly when the concentration of iron is analysed. From the beginning, in all three concentrates tested, the Fe^{3+} evolved parallel to total Fe. That is, as rapidly as Fe was produced, as a consequence of oxidation of the minerals, Fe^{2+} was transformed into Fe^{3+} through the intervention of the microorganisms. Such behaviour contributed to the leaching of copper and zinc since Fe^{3+} also acted as oxidant, attacking the ore by means of an indirect leaching mechanism according to next reaction:

$$MS + 2Fe^{3+} \rightarrow M^{2+} + 2Fe^{2+} + S^{\circ} \quad (3)$$

where M is the metal-forming part of the metallic sulphide. Both the Fe^{2+} and the S° formed in this reaction can be reoxidized by the bacteria which results in a new attack on the mineral and acidification of the medium. In the CCu concentrate, the dissolution of iron began as soon as bacterial growth and ore oxidation took place, although the relatively high pH provoked a substantial precipitation of jarosites and a reduction in the concentration of iron. The high salt concentration of the 9K medium contributes to the formation of these jarosites and to the loss of Fe^{3+} from the solution. The pH was lower in the case of the other two concentrates used and so jarosite precipitation was also lower but still present. With both these concentrates the concentration of iron in solution was higher than when CCu was used. As in the case of the cell population and pH, the amount of iron in solution rose earlier with CCuL than with the CGRT concentrate, illustrating the better bacterial adaptation to this concentrate.

The evolution of iron was totally opposite in the sterile experiments. To the lower amount of iron in solution can be added the fact that almost all the iron was in the form of Fe^{2+}. There was also a decrease in total iron during the last days of the experiments due to the precipitation of jarosites caused by the relatively high pH.

3.3. Evolution of microbial populations at 68.5°C

The biotechnological process of treating ores with thermophilic microorganisms involves costly investment at an industrial level. The use of a given bacterial species involves a certain risk since operating conditions are not as easily controlled as they are in a laboratory and it is of course impossible to sterilise the material or the ore to be used. This means that the microbial species used must be capable of adapting easily to their surroundings and of competing favourably with any indigenous microbial flora in the ore. In laboratory experiments carried out at temperatures below 50°C the autochthonous flora frequently gain the upper hand over inoculated bacteria so that any advantage that it is hoped to gain by using a specific inoculum is lost [27]. For this reason, experiments using *S. rivotincti* on unsterilized minerals for three consecutive cultures were carried out to ascertain whether any microorganism present in these solid samples upset the leaching process. The experimental conditions were optimal for growth (68.5°C, pH 2) with a pulp density of 3% of CCu, CGRT and CCuL concentrates.

The controls carried out by optical and electronic microscopy showed that during the three consecutive cultures no new type of bacteria appeared and leaching was exclu-

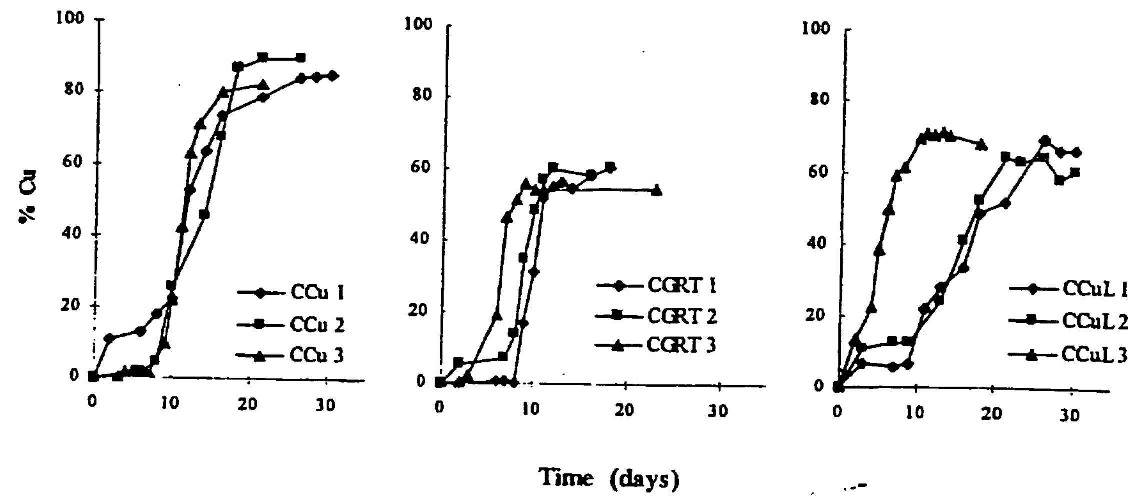

Fig. 4. Copper dissolution from the three mineral concentrates used in the experiments.

sively carried out by *S. rivotincti*. This was an important finding, which might contribute to its successful industrial application, since no competitors to inhibit its operation were found in the medium.

Figs. 4 and 5 show the results obtained when the different concentrates were bioleached. The amount of metals in solution, copper and zinc, did not increase in any significant way from one pass to the next, which means that the microorganisms were already well adapted to the process of metal extraction and that they simply had to adapt to different concentrates from which they obtained energy.

In all cases substantial amounts of metal were extracted. On the other hand, during the three consecutive cultures the progressive adaptation of the thermophilic microorganisms to the leaching process could be observed, which resulted in shorter times being necessary to obtain maximum yields. From the third culture to the first, the time needed

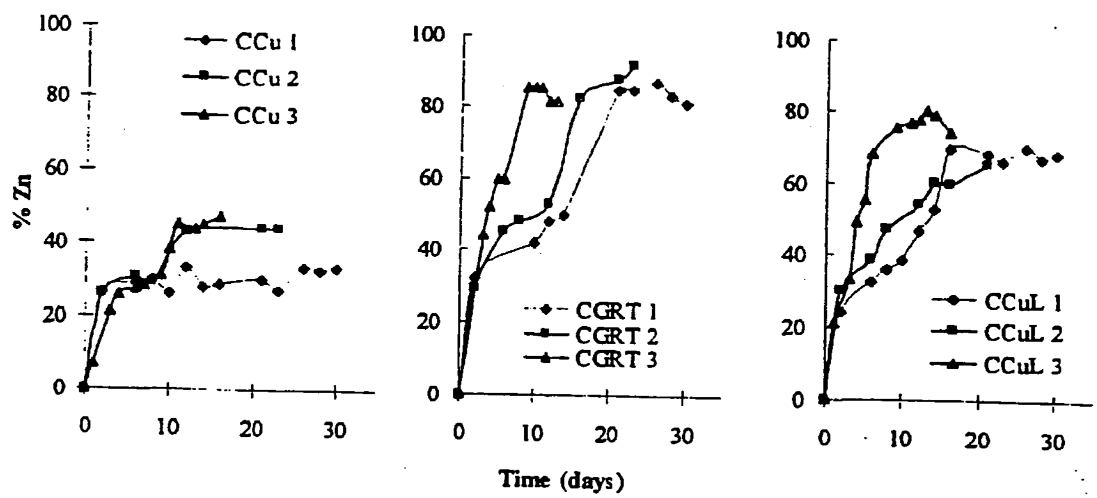

Fig. 5. Zinc dissolution from the three mineral concentrates used in the experiments.

to reach the maximum value of copper in solution was almost halved in CGRT (from 18 to 9 days) and reduced by two thirds in CCuL (from 21 to 7 days). In these two concentrates the lag phase of the first two passes, which had delayed the beginning of copper leaching, was almost entirely eliminated in the third pass. This reduction in the time necessary for the lag phase was much less in the CCu concentrate, with which the concentrations of copper in solution were the highest.

The bacterial adaptation to the ore and the corresponding reduction in time was also evident in the dissolution of zinc from the CGRT and CCuL concentrates (Fig. 5), although there was no significant increase in the maximum levels of metal in solution. In the case of the CCu concentrate, an increase in the concentration of zinc dissolved was observed as well as a reduction in time.

The pH of the different tests (Fig. 6) rapidly dropped below the initial values, particularly in the case of CCuL, where microbial activity was the greatest. As a consequence of this adaptation of the thermophilic microorganisms to the different minerals, the increase in pH due to consumption of acid by the gangue of the mineral decreased. Other authors [28,29] have shown that a long period of bacterial adaptation to the ore, using *T. ferrooxidans*, can even eliminate this increase in pH which was observed at the beginning of the leaching process. A good adaptation of the bacteria to the ore was also observed in the case of the CCu concentrate, in which the pH dropped with each new pass. All the above demonstrate the importance of microorganism adaptation to the mineral to be attacked even if the amount of metal which can be leached does not increase, as in the case of the three concentrates studied here. The reduction in the time necessary to reach maximum leaching is clear because of the relatively slow kinetics of the bioleaching process which hinder its application on an industrial scale.

Finally, new experiments with each of the three mineral concentrates with no sterilisation or inoculum were prepared. No bacteria were observed, possibly because the

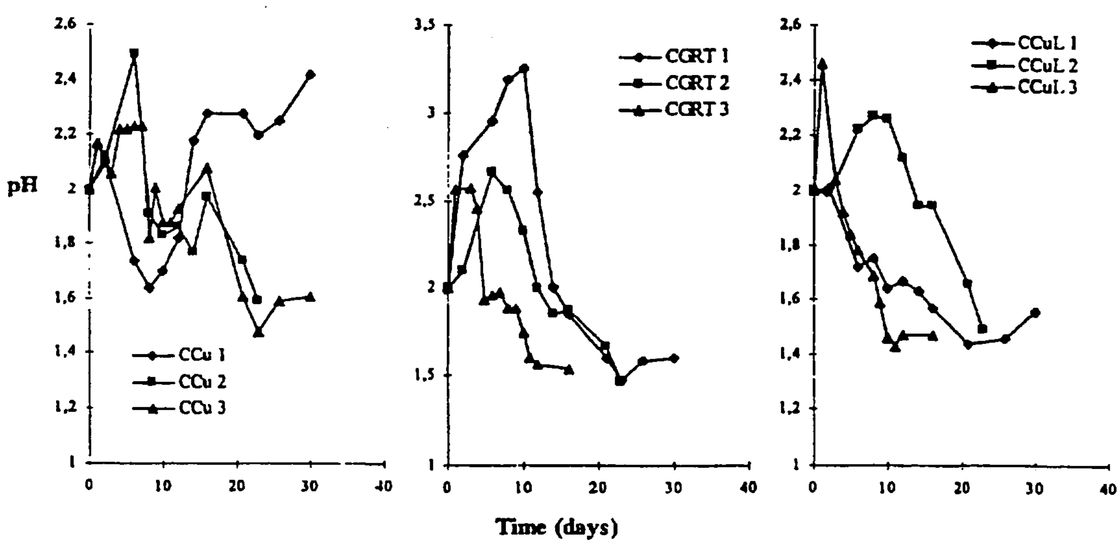

Fig. 6. Evolution of pH in the leaching of the three mineral concentrates used.

high temperature did not allow the microorganisms present in the mineral to grow. However, experiments carried out at lower temperatures (37 and 50°C) with the same concentrates revealed the development of different types of microorganisms associated with the ore alongside other heterotrophic microorganisms and fungi. However, the presence of heterotrophic bacteria and fungi might hinder the development of some species of chemolithotrophic microorganisms. *S. rivotincti*, despite its strictly chemolithotrophic metabolism, was not affected by the presence of small amounts of organic substances present in the medium. This was confirmed in the microorganism characterization tests and in experiments involving the leaching of concentrates with flotation reagents. It is possible to work with pure cultures of this bacterium without the danger of competition from autochthonous species in the mineral.

From the above results, the high capacity of *S. rivotincti* to extract copper and zinc is manifest. However, the principal interest in this microorganism lies in its ability to leach chalcopyrite concentrates with higher copper efficiency than that obtained from ores tested previously. For this reason, it was used to attack a chalcopyrite concentrate containing 27.6% of copper. In an attempt to maximise the yield, the leaching process was started in the presence of different compounds thought likely to increase the economic feasibility of the process: NaCl, or different metallic cations (Bi, Cr, Al, As, Co, Cd, Hg, Ag, Mo) acting as catalysts.

3.4. Leaching in the presence of chlorides

In many countries such as Chile, which has important chalcopyrite leaching facilities, sea water (0.32 M NaCl) may be used as the leaching medium. The action of the chloride ion on copper minerals is well known and, on chalcopyrite concentrates, produces cupric or iron chloride.

Sea water is an abundant and cheap leaching medium and so a microorganism's resistance to high concentrations of sodium chloride is of great interest. Some thermophilic microorganisms such as *S. metallicus* oxidise metallic sulphides and can grow in NaCl concentrations close to 0.5 M [11]. A series of experiments were carried out to test the capacity of *S. rivotincti* to grow at different NaCl concentrations during the leaching process. The results (Table 2) clearly show that most concentrations of NaCl were toxic for the microorganisms and only at the two lowest concentrations the microorganisms

Table 2
Results of the leaching experiments in the presence of NaCl

Medium	Final pH	Copper (%)	Iron (%)	Cells ml^{-1}
9K without NaCl	1.6	68.8	18.4	8.50×10^{8}
9K + 0.05 M	2.3	53.2	7.7	5.00×10^{5}
9K + 0.1 M	2.6	47.8	5.7	6.25×10^{5} (6)
9K + 0.25 M	3.7	44.2	1.7	1.00×10^{5} (3)
9K + 0.5 M	3.6	42.7	4.5	2.00×10^{4} (2)
9K + 0.75 M	3.6	39.5	4.1	– (1)
9K + 1 M	3.5	35.1	4.8	– (1)

The cell concentrations correspond to the maximum amount of cells observed. In parentheses, the number of days it took for the bacterial culture to disappear. The initial pH of all the experiments was 1.8 and the leaching time 9 days.

survived for a short time. In all the other tests the number of cells rapidly dropped and even at 0.5, 0.75 and 1 M they disappeared in less than 4 days.

The pH behaved in a similar way, rapidly rising with high NaCl molarities, thus hastening bacterial death, before levelling off and finally dropping. In experiments with low molarities, the pH rose much more gradually and remained at levels which the bacteria could support even though these levels were far from the optimum pH of *S. rivotincti*. However, despite these drawbacks, the amount of copper brought into solution was still much above that obtained in normal sterile experiments (without NaCl but otherwise in the same conditions as used in the tests being described) with their extraction being less than 10%. In the experiments where the bacterial culture disappeared on the first day, 30 and 40% of the copper was extracted, while in the 0.05 M experiments, in which the pH rose less and the bacteria survived longer, more than 50% of the copper was extracted. In other words, sodium chloride had a significant effect on the chalcopyrite concentrate, as can be seen in the first samples taken when the amount of copper extracted in the presence of high concentrations of NaCl was higher than in the experiments without salt. Then, due to the absence of bacteria, extraction rates remained at the same level while at the end of the experiment the concentration of copper dissolved was greater in the tests where the bacteria had survived longer. The amount of iron brought into solution was also much less in the experiments with NaCl than in the reference one. Most of the iron was precipitated in the form of iron oxides as observed from an analysis of the residues by X-ray diffraction (not shown). Very small amounts of jarosites were observed (in the experiments without chlorides and with 0.05 and 0.1 M of NaCl) since in all the other experiments the elemental sulphur, which was formed in the attack on chalcopyrite, was not oxidised to sulphate and only the sulphate ions corresponding to the 9K medium were present. The absence of these sulphates prevented the formation of jarosites. This was confirmed by the diffractograms of the residues in the tests with chloride concentrations greater than 0.2 M, which revealed the presence of small amounts of elemental sulphur.

In view of these results it could be interesting to use bacteria which are resistant to high NaCl concentrations so that their leaching properties and those of Cl^- ions may complement each other. However, such a process must be based on a long adaptation period beginning with very low NaCl concentrations since, as has been demonstrated, the *S. rivotincti* is very sensitive to chloride salts.

3.5. Leaching in the presence of different metallic cations

Some metallic cations favour bioleaching, as has been demonstrated by the use of silver in the presence of mesophilic microorganisms, or bismuth in experiments involving the thermophilic microorganism *Sulfolobus* BC [17,21]. The action of these cations seems to be purely chemical since the interchange between ion and substrate encourages the liberation of ions from the initial solid [30]. A series of experiments to ascertain the capacity of different metallic cations to favour copper extraction from a chalcopyrite concentrate in the presence of *S. rivotincti* was performed. These cations were used at concentrations below the toxicity thresholds detected for the microorganisms previously grown on the same cations. For this reason, bacterial growth was generally good in all

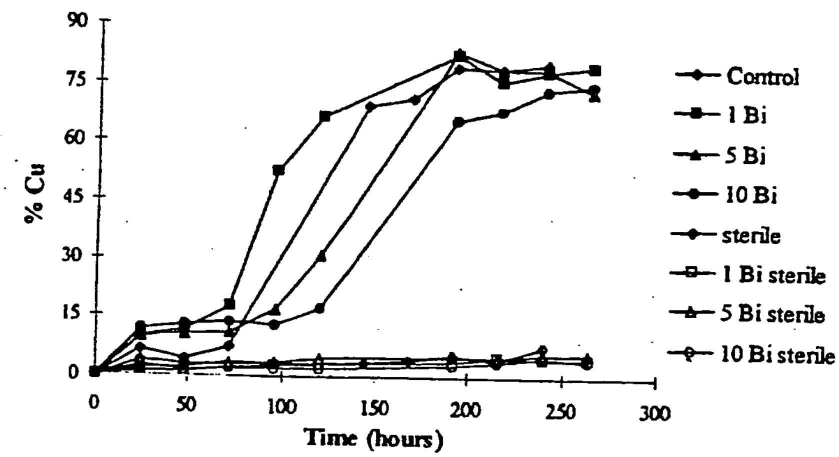

Fig. 7. Copper dissolution from the chalcopyrite concentrate in the presence of different bismuth concentrations.

similar in all the inoculated experiments but once again, the greater amount of dissolved copper was recorded in the reference and 1 g Bi (kg concentrate)$^{-1}$ experiments. The fact that the test involving bismuth had a greater concentration of copper in solution may also have been due to the greater concentration of iron which was present throughout the test since this would act as a leaching agent. Perhaps the bismuth precipitated the phosphates from the medium, thus withdrawing these anions from the solution (which otherwise might have precipitated along with the Fe^{3+} to produce $FePO_4$) and permitting a greater concentration of Fe^{3+} in solution. This, together with the higher growth of the bacterial population, led to a degree of leaching higher in this test than in the case of the reference culture. At the end of the inoculated experiments a substantial amount of jarosites had precipitated, thus withdrawing most of the iron in solution, as is reflected by the very small iron concentrations observed during analysis of the solutions.

It must be pointed out that no significant difference in the copper leaching was observed between any of the sterile tests, which means that bismuth had very little effect on these experiments. Differences in the amount of iron extracted were found in favour of the test not involving cation, which could have been due to the difference in pH produced between this test and the sterile tests with cation.

Whatever is the case, no positive effect of Bi on copper dissolution was observed, and the differences between the different tests could be better explained by differences in bacterial growth. The principal effect of bismuth was to increase the initial kinetics with respect to the reference experiment and only when the concentration was 1 g Bi (kg concentrate)$^{-1}$. Greater concentrations slowed the process down perhaps because of the lack of adaptation of the microorganisms to the cation.

3.7. Leaching in the presence of silver

Silver is another cation with a proven positive effect on chalcopyrite leaching with mesophilic microorganisms [16]. Silver interacts with the surface of chalcopyrite particles, forming silver sulphide according to the reaction [4]:

$$CuFeS_2 + 4Ag^+ \rightarrow 2Ag_2S + Fe^{2+} + Cu^{2+} \quad (4)$$

The Fe^{2+} is oxidised by the bacteria to Fe^{3+} and this reacts with the silver sulphide according to Ref. [5], liberating Ag^{+} ions again which can then react again with the chalcopyrite and the elemental sulphur which is oxidised by the bacteria [5]:

$$Ag_2S + 2Fe^{3+} \rightarrow 2Ag^{+} + 2Fe^{2+} + S^{o} \quad (5)$$

However, this improvement in chalcopyrite dissolution has not been demonstrated in the presence of thermophilic microorganisms, probably because this cation is highly toxic for most of the bacteria which have been tested [17]. Previous experiments showed that leaching with *S. rivotincti* in the presence of silver was less effective than without the cation. The action mechanism of silver is purely chemical and since the concentrations used are below the toxicity threshold, a series of tests with high Ag concentrations were carried out, as in the case of bismuth, to check the possible beneficial effect of this cation. The results are summarised in Table 5.

It can be seen that the bacterial population increased in inverse proportion to the concentration of silver used in the tests and always much less than in the reference test without cation. Whatever is the case, the concentrations of silver used were not toxic for the bacteria since their populations grew in all the tests except in the case of the highest concentration used (6 g Ag (kg concentrate)$^{-1}$).

Metal extraction was linked to the number of cell in different cultures, especially in the case of copper. There was a great difference between the inoculated experiment which served as control and those containing silver, and within this group of tests, the best results were achieved with the two lowest concentrations of cation although the levels of copper in solution were always below those in the reference experiment. In all the tests, including that using 6 g Ag (kg concentrate)$^{-1}$, the dissolution of copper was always greater than in the corresponding sterile experiments although this improvement was related to the biological capacity of the microorganisms rather than to the presence of cation. This absence of a positive effect on the part of the Ag^{+} ion can be put down to the Fe^{2+} liberated into the medium which, once oxidised to Fe^{3+} by the bacteria, was

Table 5
Results of the leaching experiments in the presence of silver after 256 h (St: sterile; Inoc: inoculated test)

	Time (h)	1 g Ag (kg ore)$^{-1}$		3 g Ag (kg ore)$^{-1}$		6 g Ag (kg ore)$^{-1}$		Reference tests	
		St.	Inoc.	St.	Inoc.	St.	Inoc.	St.	Inoc.
pH (initial)	0	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
pH (final)	256	1.8	1.4	1.7	1.5	1.7	1.6	1.8	1.3
Cell population									
Initial population	0		1.0×10^{7}		1.0×10^{7}		1.0×10^{7}		1.4×10^{7}
Final recount	256		4.8×10^{7}		3.6×10^{7}		1.2×10^{7}		4.0×10^{8}
Copper extraction									
Final dissolution (%)	256	5.1	45.2	5.8	44.5	7.8	18.1	8.0	80.0
Iron extraction									
Final dissolution (%)	256	2.0	3.0	1.4	6.1	0.7	4.4	5.1	10.4

precipitated as jarosites. These could avoid reaction [5] and even withdraw silver from the medium since this element forms very stable argentojarosites. From Table 5 it can be seen how the iron in solution was much less in the experiments with silver than without. These results were confirmed by X-ray diffraction of the residues, which revealed a massive presence of jarosites in the precipitates. The high temperature used in the tests together with the use of a sulphate rich medium (9K) favoured the precipitation of these basic iron sulphates.

4. Conclusions

S. rivotincti is an archaeobacteria with a high capacity for oxidising different mineral concentrates both of complex sulphides and chalcopyrite. It can be used with pulp densities of above 10% but only after a previous adaptation process. The microorganism is especially suitable for working as a pure culture since its natural conditions and the medium in which it develops shows that it can dominate possible autochthonous microorganisms present in the mineral which might otherwise harm the leaching process. The use of sodium chloride as a catalyst is harmful for the microorganism since it is highly toxic even at low concentrations. However, the fact that the bacterial population does not disappear when NaCl concentrations below 0.05 M are used suggest that a suitable period of adaptation might lead to a greater tolerance of chloride than that which has so far been obtained. The use of various cations to catalyse chalcopyrite dissolution had no beneficial effect. Only the addition of small amounts of bismuth increased the kinetics of the process, especially during the first phase, although even here there was no increase in the final amount of copper brought into solution. Again a suitable process of adaptation to these cations might increase the process yield. The use of a medium more dilute than 9K medium and a lower starting pH (of about 1.5) might contribute to increase copper recovery. 9K medium at pH 1.8 was used to create the optimal conditions for microorganism growth. Although it is possible that they are not the optimal conditions for bioleaching.

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